HDL and cardiovascular risk: is cholesterol in particle subclasses relevant?

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This editorial refers to ‘HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the Lipoprotein Investigators Collaborative’¹, by S.S. Martin et al. on page 22.

Dyslipidaemia is one of the major risk factors for the development of cardiovascular disease, which remains the leading cause of death in the world. Despite major advances in the treatment of dyslipidaemia, residual cardiovascular risk remains high in a significant number of patients, a fact that has prompted intensive investigation of medications raising HDL-cholesterol (HDL-C). Indeed, robust epidemiological evidence had shown that low HDL-C was inversely related to coronary outcomes in the pre-statin era, and even in patients treated aggressively with statins in the Treating-to-New-Targets (TNT) trial. However, the failure of several HDL- C-raising drugs including torcetrapib and niacin in statin-treated patients, combined with genetic studies, has recently challenged the relevance of the cholesterol content of HDL (i.e. HDL-C) (reviewed in Tujeda and Rader²). As a consequence, the focus on HDL-C has now started to shift away from a cholesterol-centric view towards alternative indexes of HDL such as particle size, subclass distribution, and measures of HDL functionality. However, this approach remains vague, and further progress is hampered by the facts that HDLs are heterogeneous particles and various HDL subfractionation methods exist, which also differ in their nomenclature, making comparisons across these methods challenging. This complexity has been compounded over the last 10 years by inconsistencies in the literature on HDL-C subclasses and function.

Martin et al. now report the analysis of HDL₂-C (larger, more buoyant) and HDL₃-C (smaller, more dense) subclasses by vertical-spin density gradient ultracentrifugation and their respective association with clinical outcomes in two cohorts of secondary prevention, namely the Translational Research Investigating Underlying disparities in acute Myocardial infarction Patient’s Health status (TRIUMPH) study of 2465 acute myocardial infarction patients, and the Intermountain Heart Collaborative Study (IHCS) study of 2414 patients who underwent coronary angiography. The parallel analysis of both cohorts showed increases > 50% in the risk both of mortality in TRIUMPH and of mortality or myocardial infarction in IHCS for the lowest tertile of HDL₂-C compared with higher tertiles in fully adjusted multivariable models. These results were modestly attenuated by further adjustment for apolipoprotein A-I (ApoA-I), the major protein in HDL. In contrast, no associations with outcomes were observed for HDL-C and HDL₃-C. Even though the observed predictive value of HDL-C subclass testing is encouraging and confirms previous data showing that HDL₂-C levels are inversely associated with cardiovascular risk, the evidence linking cholesterol concentration in specific HDL-C subpopulations to cardiovascular outcomes is anything but consistent. Indeed, HDL₂-C was inversely correlated with cardiovascular risk in the Kuopio Ischemic Heart Disease Risk Factor Study and the Quebec Cardiovascular Study. In contrast, the Monitored Atherosclerosis Regression Study (MARS) found HDL₃-C to be an independent correlate of coronary lesion progression, while no predictive value of any HDL subclass was found in the Atherosclerosis Risk in Communities (ARIC) study, the Physicians’ Health Study, and the Caerphilly and the Speedwell Collaborative Heart Disease Studies. Differences in study designs, patient populations, adjustments for confounders such as statin use, HDL-C fractionation techniques, and study outcomes may explain the inconsistencies amongst studies. While the study of Martin et al. adds to the body of data supporting an important role for HDL₂-C in cardiovascular risk prediction, it also suggests that HDL is more complex than can be captured by current technologies.

The study by Martin et al. points to an essential problem in the assessment of HDL atherogenicity, which is the lack of standardization across diverse HDL-C fractionation methodologies. In the study under discussion, HDL-C subclasses were measured by the Vertical Auto Profile (VAP) method. The VAP separates lipoproteins based on their density using single vertical-spin density gradient ultracentrifugation and reports cholesterol concentrations in total HDL-C, HDL₂-C, and HDL₃-C (Figure 1). One advantage of this method is that it has been used for HDL subfractionation in multiple epidemiological studies. However, other promising technologies have emerged, such as two-dimensional gel electrophoresis and nuclear...
magnetic resonance (NMR) spectroscopy, the latter method separating up to 26 HDL-C subpopulations by their differences in magnetic susceptibility (Figure 1). Although characterization of HDL-C subpopulations by NMR is now commonly used and has been linked to cellular cholesterol efflux, the technique awaits broader validation. At the moment, none of the currently used techniques can be viewed as markedly superior to the others. Furthermore, we lack reference standards for the measurement of lipid subclasses and, when compared head to head, substantial heterogeneity of results obtained from subclass assays is detected. Even though an attempt was recently made to unify and integrate the information produced by different HDL-C measurements, this methodological heterogeneity is definitely one of the main limitations of using HDL composition to predict cardiovascular risk. As pointed out by the authors, further investigation is required to compare different techniques and their predictive value. Importantly, before measurement of HDL-C subclasses can be applied in clinical routine, it will be critical to assess why and how specific separation methods have led to such contrasting results in the past.

Unfortunately, there is also no consensus as to the role of HDL subclass function in cardiovascular risk assessment. HDL subclass function, in particular HDL3, can be highly vulnerable to possible detrimental effects of dyslipidaemia, enhanced oxidative stress, and inflammation, and progressively lose normal biological function (potentially becoming dysfunctional). In contrast, under normal conditions, HDL3 seems to exert a more powerful protective action against LDL oxidation than HDL2 and is therefore more resistant to oxidative modification from secondary lipid peroxidation products. Similarly, HDL3 exerts more potent anti-inflammatory and cytoprotective actions than HDL2, while HDL2 shows more powerful vasodilatory and antithrombotic activities than HDL3. Given these data, the study by Martin et al. raises the following questions. (i) Is dysfunctional HDL the result or the cause of the disorder? (ii) Will a shift towards more HDL3-C and less HDL2-C improve HDL...
function and outcomes? (iii) Should pharmacological intervention target the transformation of native HDL into ‘dysfunctional’ HDL rather than simply augmenting the cholesterol content of HDL? The recent dal-ACUTE trial possibly sheds some light on HDL dysfunction by demonstrating that there is a disconnect between a pharmacologically induced increase in HDL-C levels and cholesterol efflux in patients with acute coronary syndrome. It remains speculative how HDL particles develop their proatherogenic role. The study by Martin et al. could not address this crucial issue. While there are inconsistencies regarding the proatherogenic role of HDL2-C and HDL3-C, ApoA-I has been shown to remain protective even at higher levels and may therefore represent an attractive predictor of cardiovascular risk. Accordingly, in their study, Martin et al. demonstrated a stronger positive correlation of ApoA-I with HDL3-C than with HDL2-C, and speculate that this may indicate a significant contribution of HDL3-C to the majority of HDL functions. While small HDL particles indeed seem to have a greater capacity to stimulate and promote cellular cholesterol efflux, it remains largely unknown whether HDL functions are linked to HDL size, concentration, ApoA-I composition, density, mobility, or any combination of these properties.

Do the results reported by Martin et al. mean that we should now focus on HDL3-C in clinical practice? Until it is shown that a change in HDL3-C induced by an intervention translates into favourable clinical outcomes, HDL3-C measured by VAP should be considered a potential risk marker and not a risk factor. Innovative pharmacological approaches targeted at HDL are currently under development and will help to clarify the roles of different HDL particles and functions. By recognizing the importance of HDL functionality, these new developments in lipid therapies may hold promise to achieve a reduction in cardiovascular risk. However, future studies will also have to explore which HDL-C subclasses are more prone to be dysfunctional, as well as at what stage of HDL metabolism such changes in HDL functionality are most likely to occur. As a priority for future research, standardization among different HDL-C fractionation methodologies and, in an ideal world, development of a method to subfractionate HDLs based on their functional properties should be achieved. For the time being, we should withhold the final word on the use of HDL subclasses in cardiovascular risk prediction and await further data linking their baseline characteristics and changes over time to clinical endpoints, HDL functional parameters, and, ideally, genotypes.

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